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13. ABSTRACT (Maximum 200 words) Purpose: To investigate the effects of prolonged hypoxia and antioxidant supplementation on ventilatory threshold (VT) during high-altitude (HA) exposure (4300 M). Methods: Sixteen physically fit males (25 + 5 yr; 77.8 + 8.5 kg) performed an incremental test to maximal exertion on a cycle ergometer at sea level (SL). Subjects were then matched on VO2peak, ventilatory chemosensitivity, and body mass and assigned to either a placebo (PL) or antioxidant (AO) supplement group in a randomized, double-blind manner. PL or AO (12 mg of B-carotene, 180 mg of a-tocopherol acetate, 500 mg of ascorbic acid, 100 µg of selenium, and 30 mg of zinc daily) were taken 21 d prior to and for 14 d at HA. During HA, subjects participated in an exercise program designed to achieve an energy deficit of approximately 1400 kcal.d-1. VT was reassessed on the second and ninth days at HA (HA2, HA9). Results: Peak power output (Wpeak) and VO2peak decreased (28%) in both groups upon acute altitude exposure (HA2) and were unchanged with acclimatization and exercise (HA9). Power output at VT (WVT) decreased from SL to HA2 by 41% in PL, but only 32% in AO (P<0.05), WVT increased in PL only during acclimatization (P<0.05) and matched AO at HA9. Similar results were found when VT was expressed in terms of % Wpeak and % VO2peak. Conclusions: VT decreases upon acute HA exposure but improves with acclimatization. Prior AO supplementation improves VT upon acute, but not chronic altitude exposure.				
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Changes in Ventilatory Threshold at High Altitude: Effect of Antioxidants

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¹University of Colorado at Colorado Springs, Colorado Springs, CO; ²Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; ³University of Miami, Miami, FL; ⁴University of Massachusetts, Amherst, MA; ⁵University of California Berkeley, Berkeley, CA; and ⁶U.S. Army Research Institute of Environmental Medicine, Natick, MA

ABSTRACT

SUBUDHI, A. W., K. A. JACOBS, T. A. HAGOBIAN, J. A. FATTOR, S. R. MUZA, C. S. FULCO, A. CYMERMAN, and A. L. FRIEDLANDER. Changes in Ventilatory Threshold at High Altitude: Effect of Antioxidants. *Med. Sci. Sports Exerc.*, Vol. 38, No. 8, pp. 1425–1431, 2006. **Purpose:** To investigate the effects of prolonged hypoxia and antioxidant supplementation on ventilatory threshold (VT) during high-altitude (HA) exposure (4300 m). **Methods:** Sixteen physically fit males (25 ± 5 yr; 77.8 ± 8.5 kg) performed an incremental test to maximal exertion on a cycle ergometer at sea level (SL). Subjects were then matched on $\dot{V}O_{2\text{peak}}$, ventilatory chemosensitivity, and body mass and assigned to either a placebo (PL) or antioxidant (AO) supplement group in a randomized, double-blind manner. PL or AO (12 mg of β -carotene, 180 mg of α -tocopherol acetate, 500 mg of ascorbic acid, 100 μ g of selenium, and 30 mg of zinc daily) were taken 21 d prior to and for 14 d at HA. During HA, subjects participated in an exercise program designed to achieve an energy deficit of approximately $1400 \text{ kcal} \cdot \text{d}^{-1}$. VT was reassessed on the second and ninth days at HA (HA2, HA9). **Results:** Peak power output (W_{peak}) and $\dot{V}O_{2\text{peak}}$ decreased (28%) in both groups upon acute altitude exposure (HA2) and were unchanged with acclimatization and exercise (HA9). Power output at VT (W_{VT}) decreased from SL to HA2 by 41% in PL, but only 32% in AO ($P < 0.05$). W_{VT} increased in PL only during acclimatization ($P < 0.05$) and matched AO at HA9. Similar results were found when VT was expressed in terms of % W_{peak} and % $\dot{V}O_{2\text{peak}}$. **Conclusions:** VT decreases upon acute HA exposure but improves with acclimatization. Prior AO supplementation improves VT upon acute, but not chronic altitude exposure. **Key Words:** HYPOXIA, FREE RADICAL, $\dot{V}O_{2\text{max}}$, ANAEROBIC THRESHOLD, LACTATE THRESHOLD

Incremental exercise tests to maximal exertion are commonly used to assess training status of individuals. Although peak aerobic capacity ($\dot{V}O_{2\text{peak}}$) is a good overall predictor of performance among populations of varied fitness levels, it fails to predict performance among more homogeneous groups of trained individuals (4). Several authors have reported that nonlinear increases in physiological variables, such as blood lactate, HR, and ventilation observed during progressive exercise tests represent “thresholds” that are better predictors of performance than $\dot{V}O_{2\text{peak}}$ (4) and accurately reflect work rates during actual competition (16).

At high altitude (HA), aerobic exercise performance is substantially compromised due to low ambient partial pressure of oxygen. Decrements in performance are greatest upon initial exposure to hypoxia, yet improve with acclimatization (3,12,17). Because physiological thresholds are

associated with prediction of performance (4), it may seem logical that the lactate response to exercise follows a similar pattern of acclimatization (11); however, the mechanisms responsible for decreased blood lactate concentrations during exercise following acclimatization remain paradoxical in light of persistent hypoxemia (24). Given the complexities of lactate kinetics during exercise at altitude, investigation of other related variables (e.g., ventilation, HR) has attracted attention.

Lactate ions (La^-) are exported simultaneously with protons (H^+) across myocyte membranes; thus, their accumulation in blood is well correlated during exercise (27). Because H^+ and CO_2 are potent simulators of ventilation, nonlinear increases in ventilation (i.e., ventilatory threshold (VT)) during incremental exercise tests have been shown to be noninvasive alternatives to lactate threshold (LT) determinations (28). The mechanistic relationship between VT and LT is a topic of great theoretical debate; yet, from a practical standpoint, both variables have been used successfully to longitudinally assess training status and performance. Recently, Amann et al. (1) evaluated several methods of assessing performance thresholds in a group of trained individuals and reported that VT was the most accurate and reliable index of laboratory cycling time-trial performance. Additionally, Lucia et al. (16) reported that VT was the only physiological variable obtained during an incremental exercise test that was related to time-trial performances during the Tour de France.

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Despite the advantages of VT assessments, there have been few measurements of VT at HA. Previous studies have focused exclusively on the VT response to acute, but not chronic hypoxia. Breathing reduced oxygen concentrations ($F_{I}O_2$, 12–14%) during incremental exercise tests at low altitude (8,13,21) and ascent from sea level (SL) to 4300 m (6) has been shown to decrease VT by 18–33%. Such results parallel changes observed in both LT (7,14,18) and exercise performance (3,9) upon ascent to HA; however, to our knowledge, no studies have described changes in VT over a longer period of time to see whether VT follows a similar pattern of improvement with acclimatization.

Strategies that improve physical performance under hypoxic conditions are of great interest to those who work, exercise, or compete at HA. Increased free radical production at HA has been suggested to be responsible for decrements in physical performance (2). Such findings imply that scavenging free radicals with supplemental antioxidants (AO) may improve exercise performance at altitude, yet only one study has tested the effect of AO supplementation on physical performance over an extended stay at HA. Simon-Schnass and Pabst (25) studied the effects of vitamin E in a group of HA mountaineers over the course of a 10-wk expedition. After 4 wk at 5000 m, subjects taking 400 mg·d⁻¹ of α -tocopherol acetate exhibited lower concentrations of oxidative stress markers and a higher LT than those taking placebo. Unfortunately, the study did not assess acute changes in LT over the acclimatization period and used an absolute blood lactate concentration (4.0 mmol·L⁻¹) to identify LT, a method inherently confounded by the lactate paradox of HA (24). Thus, the present investigation sought to investigate the effects of AO supplementation on VT over the first 2 wk of exposure to 4300 m. We hypothesized that VT would be decreased upon acute altitude exposure and improved with acclimatization and that AO supplementation would have an ergogenic effect of increasing VT at HA.

METHODS

Subjects. Eighteen physically fit, altitude-naïve men were recruited from Stanford University and the surrounding areas (Palo Alto/San Jose, CA) by flyers and advertisements in local newspapers. Written informed consent was obtained from all subjects following the institutional guidelines of Stanford University and the U.S. Army Research Institute of Environmental Medicine (USARIEM). All subjects were nonsmokers in good overall health as determined by health history questionnaire and medical evaluation. Subjects participated in regular endurance (cycle) exercise (> 6 h·wk⁻¹), had maximal oxygen uptake ($\dot{V}O_{2max} \geq 50$ mL·kg⁻¹·min⁻¹), were able to cycle for 1 h at 70% of peak wattage (W_{peak}), and had normal body fat composition (5–16%).

Sea-level phase. Subjects completed a 7-d diet and weight-stabilization period prior to 5 d of baseline testing at the clinical studies unit in the VA Palo Alto Health Care

System (Palo Alto, CA, 15-m elevation, atmospheric pressure 748–762 torr). On the second day of sea-level (SL) residence, subjects performed an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport, The Netherlands). Following 10 min of warm-up at a self-selected work rate, subjects cycled at 50, 100, and 150 W for 2 min each. Work rate was then increased by 30 W every 2 min until exhaustion. Objective criteria for determining maximal effort included at least two of the following: 1) increased work rate without corresponding increase in $\dot{V}O_2$, 2) respiratory exchange quotient ≥ 1.10 , 3) a pedal cadence ≤ 50 rpm. During the test, ventilation, expired gas concentrations, and HR were analyzed and averaged over 30-s periods using an automated system (Parvomedics TrueMax 2400, Consentius Technologies, Sandy, UT). $\dot{V}O_{2peak}$ was determined as the highest 30-s value obtained. VT was identified as the first systematic increase in ventilatory equivalents of oxygen ($\dot{V}E/\dot{V}O_2$) without a concomitant change in ventilatory equivalents of carbon dioxide ($\dot{V}E/\dot{V}CO_2$), as described previously (1). Peak power output was determined using methods described by Kuipers et al. (15). Three weeks prior to the HA phase, subjects were matched on age, body mass, $\dot{V}O_{2peak}$, body fat percentage, partial pressure of end-tidal CO_2 , and hypoxic ventilatory response (as described in Muza et al. (20)) and assigned to either an AO supplement or placebo (PL) group in a double-blind fashion. Based on previous research (22), a broad-based AO supplement consisting of vitamins and minerals was formulated for this study. Subjects were instructed to ingest supplements twice daily, once in the morning and once in the evening, continuing throughout the HA phase. The daily AO dose consisted of 12 mg of β -carotene, 500 mg of ascorbic acid, 180 mg of α -tocopherol acetate, 100 μ g of selenium, and 30 mg of zinc. The PL was an identical-looking and -tasting cellulose capsule.

HA phase. Approximately 6 wk after the SL phase, subjects were flown to Colorado Springs, CO (1850 m), where they spent the night in an apartment breathing supplemental oxygen. Oxygen saturation was monitored periodically by pulse oximetry (Nonin 8500, Nonin Medical, Plymouth, MN) and maintained at approximately 97%. The next morning, subjects' oxygen saturation levels were sustained via bottled 100% O_2 while they were driven to the USARIEM Maher Memorial Altitude Laboratory on the summit of Pikes Peak, CO (4300 m, atmospheric pressure 458–464 torr). Subjects resided at HA for 14 d while participating in an exercise program designed to increase total energy expenditure to approximately 1400 kcal·d⁻¹ over SL values (40% of caloric intake). Subjects were monitored and coached to achieve daily energy expenditure goals using daily activity logs accounting for each 24-h period in 15-min intervals via the Harris-Benedict equation and appropriate activity factors. Dietary intake was monitored and adjusted, depending on daily energy expenditure, to maintain an energy deficit (~ 1400 kcal·d⁻¹) common to HA expeditions (29). Subjects were fed a

standardized diet throughout the study composed of whole foods (e.g., pasta, bagels, cereal) with modest levels of AO (% RDA values: 154% vitamin A, 111% vitamin C, 107% vitamin E, 100% zinc, 256% selenium). VT tests were repeated at HA on days 2 (HA2; 24 h of exposure) and 9 (HA9; 192 h of exposure) following a 24-h rest period.

Blood draws. Resting blood samples were obtained by venipuncture to assess plasma concentrations of a limited subset of AO contained in the supplement (α -tocopherol and β -carotene), markers of oxidative stress (plasma lipid hydroperoxides, whole-blood reduced and oxidized glutathione, glutathione peroxidase activity, and urinary 8-hydroxydeoxyguanosine), and red cell hemoglobin (Hb) concentration as described previously (26).

Other assessments. As reported by Fulco et al. (9), the overall study design incorporated 720-kJ cycling time-trial tests at SL and HA to evaluate the effect of a carbohydrate versus noncaloric drink solution on exercise performance. Time trials were performed 24 h after VT tests with a 7-d washout period between trials. Because only eight subjects ingested the noncaloric drink solution during the time trials, the sample size was insufficient to test for effects of AO on cycling time-trial performance ($N = 4$ AO vs $N = 4$ PL). A *post hoc* power analysis, using effect sizes calculated from respective time-trial data, indicated that 18 subjects ($N = 9$ AO vs $N = 9$ PL) would have been necessary to detect a significant group \times time interaction (80% power with $\alpha = 0.05$). With respect to this limitation, data from the time-trial tests were used as markers of submaximal performance at SL and altitude for qualitative comparisons only.

Statistical analysis. All dependent variables were evaluated for normality (skewness, kurtosis, and normal Q-Q plots) to ensure assumptions were met for parametric statistical analysis. Repeated-measures ANOVA was used to test for differences between groups (AO vs PL) over time (SL, HA2, HA9) ($\alpha = 0.05$). Variables with significant group \times time interactions were subjected to *post hoc* analysis using *t*-tests with Holm adjustments for multiple comparisons. Data are reported as mean \pm SEM.

RESULTS

Data from two subjects were excluded from the analysis because they did not complete all three VT tests. Thus, data from 16 subjects (25 ± 1 yr; 77.8 ± 2.1 kg; 178.1 ± 1.0 cm) were analyzed ($N = 7$ PL, $N = 9$ AO). As previously reported (26), subjects lost an average of 4.3 ± 1.2 kg of body mass during the 14-d altitude exposure. Weight loss was similar between AO and PL groups while eating a diet consisting of $67 \pm 5\%$ carbohydrate, $22 \pm 5\%$ fat, and $11 \pm 1\%$ protein. The specific AO micronutrient content of the diet was formulated to just meet RDA guidelines and averaged (\pm SD) 1388 ± 302 μ g of vitamin A, 99 ± 12 mg of vitamin C, 16 ± 5 mg of vitamin E, 149 ± 27 μ g of selenium, and 11 ± 2 mg of zinc. Antioxidant supplementation

increased plasma concentrations of respective lipid-soluble vitamin concentrations (43% increase in α -tocopherol; 132% increase in β -carotene), but did not have an effect on blood-borne markers of oxidative stress (26). Red cell Hb concentration increased progressively ($P < 0.05$) from 158.8 ± 3.9 mg-dL⁻¹ at SL to 167.4 ± 3.3 mg-dL⁻¹ at HA1 and 176.6 ± 4.0 mg-dL⁻¹ at HA13, but was not affected by AO.

Sea level. At SL, subjects achieved an average peak power output (W_{peak}) of 354 ± 14 W, which was associated with a maximal oxygen consumption ($\dot{V}O_{2\text{peak}}$) of 4.36 ± 0.15 L·min⁻¹, ventilation ($\dot{V}E_{\text{peak}}$) of 161 ± 6 L·min⁻¹, and HR (HR_{peak}) of 185 ± 2 bpm (Fig. 1). Power output at VT (W_{VT}) was 244 ± 14 W ($69 \pm 2\%$ W_{peak}), equating to a $\dot{V}O_2$ ($\dot{V}O_{2\text{VT}}$) of 3.16 ± 0.14 L·min⁻¹ ($72 \pm 2\%$ $\dot{V}O_{2\text{peak}}$) and HR (HR_{VT}) of 157 ± 3 bpm ($85 \pm 2\%$ HR_{peak}) (Figs. 2–4). There were no differences between groups.

HA2. Upon acute altitude exposure (24 h), there was a 28% decrease in maximal exercise capacity, as defined by W_{peak} and $\dot{V}O_{2\text{peak}}$ (Fig. 1), which was coupled with a 7% increase in $\dot{V}E_{\text{peak}}$ (173 ± 7 L·min⁻¹) and a 5% decrease in HR_{peak} (176 ± 2 bpm). There were no differences between groups. Variables associated with VT (W_{VT} , $\dot{V}O_{2\text{VT}}$, and HR_{VT}) were lower at HA2 compared with SL for both groups (Figs. 2 and 4). Significant interactions between groups over time revealed that absolute W_{VT} (169 ± 9 vs 140 ± 10 W), relative W_{VT} (65 ± 2 vs $56 \pm 2\%$ W_{peak}), and relative $\dot{V}O_{2\text{VT}}$ (75 ± 2 vs $68 \pm 2\%$ $\dot{V}O_{2\text{peak}}$) were greater for AO versus PL, respectively (Figs. 2 and 3).

HA9. After 9 d at HA, maximal exercise capacity remained 28% lower than SL and unchanged from HA2 (Fig. 1). $\dot{V}E_{\text{peak}}$ (177 ± 5 L·min⁻¹) was greater than SL but not different from HA2. HR_{peak} (157 ± 3 bpm) was lower than both SL and HA2. There were no differences between groups in $\dot{V}O_{2\text{peak}}$, $\dot{V}E_{\text{peak}}$, and HR_{peak} . At VT, W_{VT} and $\dot{V}O_{2\text{VT}}$ remained lower than SL but had improved from HA2, due to significant changes in the PL group (175 ± 11 W and 2.37 ± 0.14 L·min⁻¹, respectively) (Figs. 2 and 4). W_{VT} and $\dot{V}O_{2\text{VT}}$ in the AO group were unchanged from HA2 (164 ± 10 W and 2.33 ± 0.12 L·min⁻¹, respectively). In relative terms, W_{VT} improved significantly from HA2, again largely due to changes in the PL group ($65 \pm 2\%$ W_{peak}) because AO values were unchanged ($67 \pm 2\%$ W_{peak}). Relative $\dot{V}O_{2\text{VT}}$ ($75 \pm 2\%$ $\dot{V}O_{2\text{peak}}$) was slightly greater than SL and HA2, but not different between groups (Fig. 3). HR_{VT} (139 ± 3 bpm) was lower than both SL and HA2, but similar between groups. Relative HR_{VT} ($88 \pm 1\%$ HR_{peak}) was not different from SL or HA2.

DISCUSSION

The goal of this investigation was to determine the effects of prolonged hypobaric hypoxia and AO supplementation on VT. We found that 1) VT was decreased upon acute altitude exposure to 4300 m, yet improved with

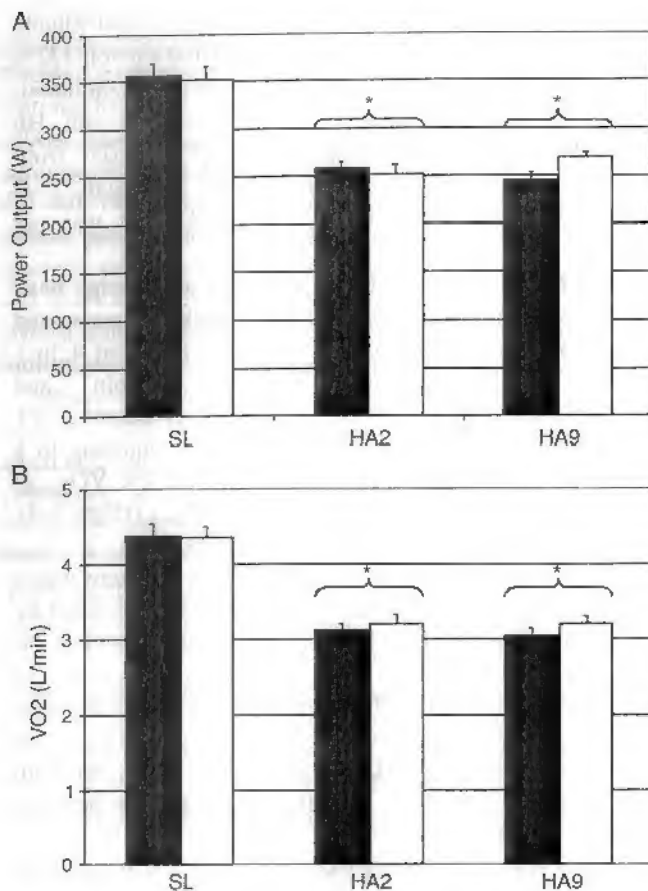


FIGURE 1—Peak exercise capacity at sea level and high altitude (4300 m). Values are mean \pm SEM for AO ($N = 9$) and PL ($N = 7$). Peak wattage (A) and $\dot{V}O_2$ (B) decreased 28% upon acute exposure to 4300 m and were not changed with acclimatization and training. There were no differences between groups (AO, solid bars; PL, open bars). *Different from SL; †different from HA9; ‡difference between groups ($P < 0.05$).

9 d of acclimatization, and that 2) AO supplementation improved VT upon acute, but not prolonged HA exposure.

The finding that peak exercise capacity was decreased approximately 28% upon ascent to HA is in line with several other investigations performed at 4300 m (10). Although $\dot{V}O_{2peak}$ is commonly used as an index for physical performance at altitude, it does not accurately reflect changes in submaximal exercise performance typically reported. Fulco et al. (9) reported a 73% decrease in cycling time-trial performance in our subject pool on the third day at altitude. Others have shown a similar mismatch between peak exercise capacity and submaximal exercise performance in shorter time trials (3) and open-ended tests to exhaustion at 4300 m (12,17). Recent studies have demonstrated that power output at VT is a more accurate measure of actual physical performance than $\dot{V}O_{2peak}$ (1,16). Our findings show that acute altitude exposure had a greater effect on power output at VT (41% decrease in PL group) than on peak power output or $\dot{V}O_2$ (28% decrease); however, both assessments appeared to underestimate changes in average power output during time-trial performance at HA (73% decrease).

The decrement in VT at 4300 m was comparable with other studies that reduced the fraction of inspired oxygen (F_{IO_2} 12–14%) during incremental exercise tests (8,13,21). Our study design most closely resembled that of Brutsaert et al. (6), who measured VT in a group of SL natives after approximately 10 h of exposure to 4338 m. They reported a 27.8% drop in $\dot{V}O_2$ at VT, which was similar to our 29.7% decrease in $\dot{V}O_2$ at VT after 24 h of exposure to 4300 m. In both studies, VT was defined as a disproportional increase in $\dot{V}E$ relative to $\dot{V}O_2$. Because the $\dot{V}O_2$ versus work-rate relationship was unaffected by acute altitude exposure, the earlier appearance of VT was due to increased submaximal $\dot{V}E$. Our results are also comparable with changes reported in LT upon acute exposure to altitude (7,14,18). We believe that increased sympathetic activity (sympathoadrenal release of epinephrine) and glycolytic flux at a given work rate (5) accelerated efflux of La^- and H^+ , which subsequently increased ventilatory drive upon acute altitude exposure. Whereas such a proposal suggests a high correlation between LT and VT, discrepancies in the temporal appearance of the thresholds (13,21) have yet to be reconciled.

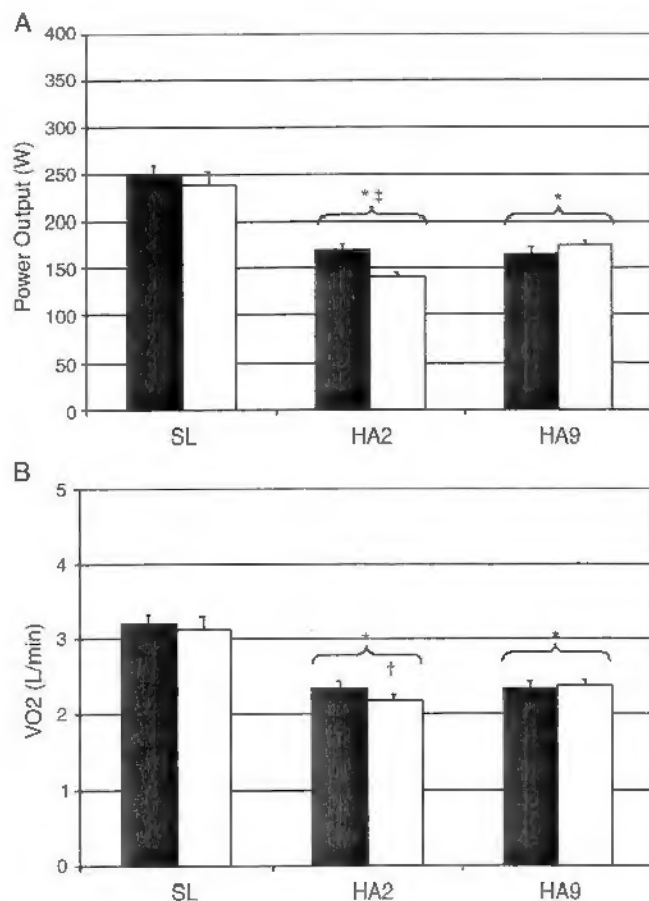


FIGURE 2—Absolute work rates at ventilatory threshold (VT) at sea level and high altitude (4300 m). Values are mean \pm SEM for AO ($N = 9$) and PL ($N = 7$). VT (A) decreased upon acute exposure to 4300 m in both groups (AO, solid bars; PL, open bars); however, the decrease in power output (B) was attenuated in AO. After 9 d, power output at VT improved in PL only. *Different from SL; †different from HA9; ‡difference between groups ($P < 0.05$).

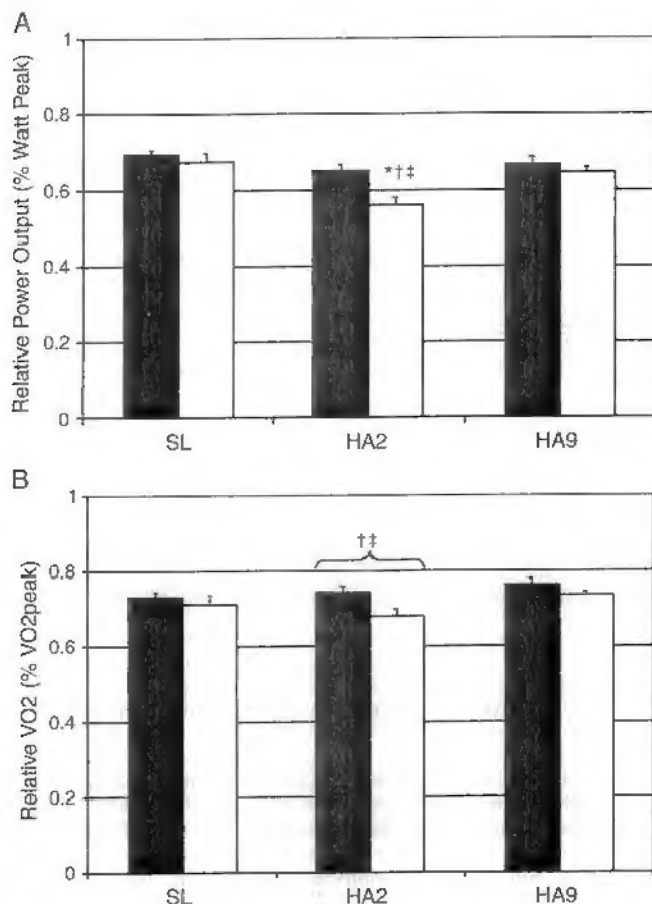


FIGURE 3—Relative work rates at ventilatory threshold at sea level and high altitude (4300 m). Values are mean \pm SEM for AO ($N = 9$) and PL ($N = 7$). Relative power output (A) and $\dot{V}O_2$ (B) at VT decreased upon acute exposure to 4300 m in PL, but returned to SL values after 9 d of HA acclimatization and training. Antioxidant supplementation mitigated changes in relative power output and $\dot{V}O_2$ at VT over the course of the study (AO, solid bars; PL, open bars). *Different from SL; †different from HA9; ‡difference between groups ($P < 0.05$).

The most remarkable finding of our study was that AO supplementation attenuated the decrease in power output at VT upon acute altitude exposure ($P < 0.05$). Whereas subjects in the PL group exhibited a 41% decrease in power output at VT, those on AO showed only a 32% decrement. Similar results were found in terms of % W_{peak} and % $\dot{V}O_{2peak}$, supporting the effect of the AO supplement. The latter appearance of VT was suggestive of an ergogenic effect of AO on acute altitude exposure. The exact mechanism of action responsible for greater power output in the AO group upon acute altitude exposure could not be determined in this field study. Although increased free radical production upon acute exposure to HA has been associated with increased energy metabolism, ultraviolet light exposure and catecholamine autooxidation (2), our results did not suggest a specific interaction between AO supplementation and indicators of free radical damage. We previously reported (26) that markers of oxidative stress (plasma lipid hydroperoxides and urinary 8-hydroxydeoxyguanosine) were not affected during the first 5 d of HA exposure; however, supplementation did increase plasma concentrations of α -tocopherol and β -carotene. The

effect of the AO supplement may have been exerted directly within muscle tissue (19), and thus was biochemically undetectable with the indirect assessments of oxidative damage used in this study. Our results may be related to those of Mohanraj et al. (19), who showed that impairment of muscle function (~50% decrease in peak tension), caused by increased free radical production under hypoxic conditions, was attenuated (~18% improvement) by AO treatment of N-acetyl-L-cysteine. Alternatively, AO may have affected redox regulation of peripheral chemoreceptors (23); however, our data did not support an effect of AO on hypoxic ventilatory responses (data to be presented elsewhere). Our findings indicate that AO affect key performance thresholds under acute hypoxia conditions and warrant further investigations to determine specific mechanisms of action.

The finding that acclimatization improved VT approximately 25% (PL group) without a change in $\dot{V}O_{2peak}$ was novel, yet followed the pattern of improvement that Fulco et al. (9) reported for our subject pool in time-trial performance (14.4% improvement). Our results are also similar to those previously reported for changes in submaximal exercise performance (3,12,17) and lactate versus work-rate relationships (11) during acclimatization. We believe the reason for the delayed appearance of VT following acclimatization was related to the lactate paradox of altitude (24), which raises the question of why muscle lactate efflux is reduced after acclimatization (2–3 wk) despite persistent hypoxemia. Again, whereas slight differences exist between the net accumulation of lactate and H^+ in blood during exercise, we believe they both follow a similar pattern of acclimatization that may reflect sympathoadrenal release of epinephrine (24). Additionally, we observed an 11% increase in erythrocyte Hb over the acclimatization period. Increased Hb may have improved nonbicarbonate buffering capacity and delayed the appearance of VT; however, changes in Hb were similar between AO and PL over the course of the study and, thus, cannot explain differences observed between groups over time.

AO supplementation did not have a measurable effect on VT after 9 d at altitude. Despite a 25% increase in PL VT, AO VT was unchanged and no longer different from PL after the acclimatization period. These results are similar to those of Simon-Schnass and Pabst (25), who reported no difference in LT after 15 d of supplementation with either 400 mg·d⁻¹ α -tocopherol acetate or placebo at 5000 m. Although the assessment of physiological effects of each supplemental AO used in this study was limited, it is possible that the acute effect of the combination of AO became masked by pronounced changes in sympathoadrenal responses over the acclimatization period (24).

An additional limitation of our study was the inability to separate independent effects of acclimatization and the exercise program on VT. However, the design was reflective of typical physical demands placed on individuals traveling for extended periods to altitude (22). Given these limitations, the slight increase in relative $\dot{V}O_2$ at VT (% $\dot{V}O_{2peak}$) over the course of the study (i.e., SL to HA9) was only mildly

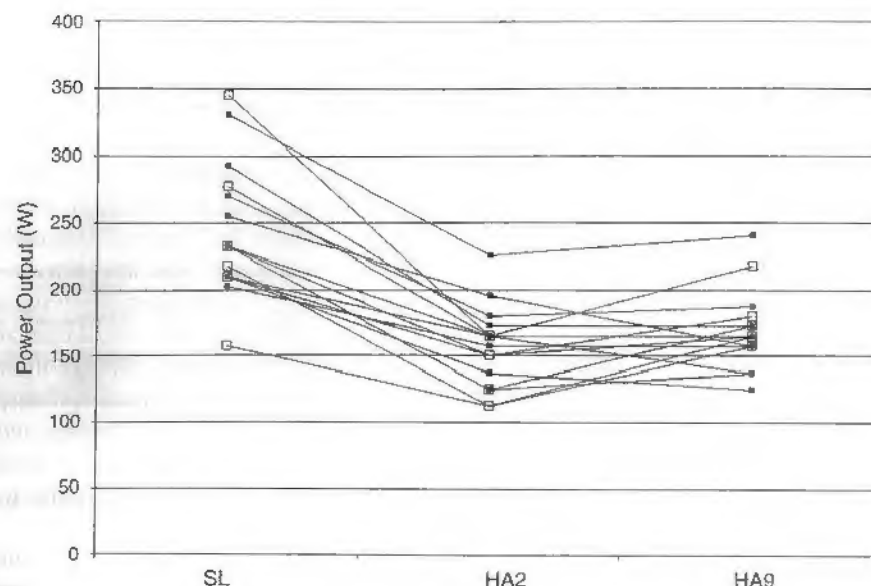


FIGURE 4—Individual work rates at ventilatory threshold at sea level and high altitude (4300 m). AO ($N = 9$) (solid squares) and PL ($N = 7$) (open squares).

suggestive of an exercise training effect because neither relative power ($\% W_{peak}$) nor relative HR ($\% HR_{peak}$) at VT was improved. Thus, we believe that the main effect on VT was due to acclimatization and not the exercise program.

CONCLUSIONS

VT was significantly decreased upon acute altitude exposure, but improved with acclimatization. Changes in VT followed patterns previously described for submaximal exercise performance and LT at altitude. Prior AO supplementation improved variables associated with VT upon acute altitude exposure, but did not have effects after a 9-d acclimatization period. Potential effects of AO on physical performance upon acute altitude exposure warrant further investigation.

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REFERENCES

1. AMANN, M., A. W. SUBUDHI, and C. FOSTER. Predictive validity of ventilatory and lactate thresholds for cycling time trial performance. *Scand. J. Med. Sci. Sports* 16:27-34, 2006.
2. ASKEW, E. W. Work at high altitude and oxidative stress: antioxidant nutrients. *Toxicology* 180:107-119, 2002.
3. BEIDLEMAN, B. A., S. R. MUZA, C. S. FULCO, et al. Intermittent altitude exposures improve muscular performance at 4,300 m. *J. Appl. Physiol.* 95:1824-1832, 2003.
4. BOSQUET, L., L. LEGER, and P. LEGROS. Methods to determine aerobic endurance. *Sports Med.* 32:675-700, 2002.
5. BROOKS, G. A., E. E. WOLFEL, B. M. GROVES, et al. Muscle accounts for glucose disposal but not blood lactate appearance during exercise after acclimatization to 4,300 m. *J. Appl. Physiol.* 72:2435-2445, 1992.
6. BRUTSAERT, T. D., E. J. PARRA, M. D. SHRIVER, et al. Spanish genetic admixture is associated with larger $V(O_2)$ max decrement from sea level to 4338 m in Peruvian Quechua. *J. Appl. Physiol.* 95: 519-528, 2003.
7. FRIEDMANN, B., T. BAUER, E. MENOLD, and P. BARTSCH. Exercise with the intensity of the individual anaerobic threshold in acute hypoxia. *Med. Sci. Sports Exerc.* 36:1737-1742, 2004.
8. FUKUOKA, Y., M. ENDO, Y. OISHI, and H. Ikegami. Chemoreflex drive and the dynamics of ventilation and gas exchange during exercise at hypoxia. *Am. J. Respir. Crit. Care Med.* 168: 1115-1122, 2003.
9. FULCO, C. S., K. W. KAMBIS, A. L. FRIEDLANDER, P. B. ROCK, S. R. MUZA, and A. CYMERMAN. Carbohydrate supplementation improves time-trial cycle performance during energy deficit at 4300 m altitude. *J. Appl. Physiol.* 99:867-876, 2005.
10. FULCO, C. S., P. B. ROCK, and A. CYMERMAN. Maximal and submaximal exercise performance at altitude. *Aviat. Space Environ. Med.* 69:793-801, 1998.
11. GRASSI, B., M. MARZORATI, B. KAYSER, et al. Peak blood lactate and blood lactate vs. workload during acclimatization to 5,050 m and in deacclimatization. *J. Appl. Physiol.* 80:685-692, 1996.
12. HORSTMAN, D., R. WEISKOPF, and R. E. JACKSON. Work capacity during 3-wk sojourn at 4,300 m: effects of relative polycythemia. *J. Appl. Physiol.* 49:311-318, 1980.
13. HUGHSON, R. L., H. J. GREEN, and M. T. SHARRATT. Gas exchange, blood lactate, and plasma catecholamines during incremental exercise in hypoxia and normoxia. *J. Appl. Physiol.* 79: 1134-1141, 1995.

14. IBANEZ, J., R. RAMA, M. RIERA, M. T. PRATS, and L. PALACIOS. Severe hypoxia decreases oxygen uptake relative to intensity during submaximal graded exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 67:7-13, 1993.
15. KUIPERS, H., F. T. VERSTAPPEN, H. A. KEIZER, P. GEURTEN, and G. VAN KRANENBURG. Variability of aerobic performance in the laboratory and its physiologic correlates. *Int. J. Sports Med.* 6:197-201, 1985.
16. LUCIA, A., J. HOYOS, M. PEREZ, A. SANTALLA, C. P. EARNEST, and J. L. CHICHARRO. Which laboratory variable is related with time trial performance time in the Tour de France. *Br. J. Sports Med.* 38:636-640, 2004.
17. MAHER, J., L. JONES, and L. HARTLEY. Effects of high-altitude exposure on submaximal endurance capacity of men. *J. Appl. Physiol.* 37:895-898, 1974.
18. McLELLAN, T., I. JACOBS, and W. LEWIS. Acute altitude exposure and altered acid-base states. I. Effects on the exercise ventilation and blood lactate responses. *Eur. J. Appl. Physiol. Occup. Physiol.* 57:435-444, 1988.
19. MOHANRAJ, P., A. J. MEROLA, V. P. WRIGHT, and T. L. CLANTON. Antioxidants protect rat diaphragmatic muscle function under hypoxic conditions. *J. Appl. Physiol.* 84:1960-1966, 1998.
20. MUZA, S. R., P. B. ROCK, C. S. FULCO, et al. Women at altitude: ventilatory acclimatization at 4,300 m. *J. Appl. Physiol.* 91:1791-1799, 2001.
21. OZCELIK, O., and H. KELESTIMUR. Effects of acute hypoxia on the estimation of lactate threshold from ventilatory gas exchange indices during an incremental exercise test. *Physiol. Res.* 53:653-659, 2004.
22. PFEIFFER, J. M., E. W. ASKEW, D. E. ROBERTS, et al. Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. *Wilderness Environ. Med.* 10:66-74, 1999.
23. POKORSKI, M., and M. MARCZAK. Ascorbic acid enhances hypoxic ventilatory reactivity in elderly subjects. *J. Int. Med. Res.* 31:448-457, 2003.
24. REEVES, J. T., E. E. WOLFEL, H. J. GREEN, et al. Oxygen transport during exercise at altitude and the lactate paradox: lessons from Operation Everest II and Pikes Peak. *Exerc. Sport Sci. Rev.* 20:275-296, 1992.
25. SIMON-SCHNASS, I., and H. PABST. Influence of vitamin E on physical performance. *Int. J. Vitam. Nutr. Res.* 58:49-54, 1988.
26. SUBUDHI, A. W., K. A. JACOBS, T. A. HAGOBIAN, et al. Antioxidant supplementation does not attenuate oxidative stress at high altitude. *Aviat. Space Environ. Med.* 75:881-888, 2004.
27. VAN HALL, G., J. A. CALBET, H. SONDERGAARD, and B. SALTIN. The re-establishment of the normal blood lactate response to exercise in humans after prolonged acclimatization to altitude. *J. Physiol.* 536:963-975, 2001.
28. WASSERMAN, K. Determinants and detection of anaerobic threshold and consequences of exercise above it. *Circulation* 76:VI29-VI39, 1987.
29. WESTERTEP, K. R. Energy and water balance at high altitude. *News Physiol. Sci.* 16:134-137, 2001.